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## **Prions, prionoids and protein misfolding disorders**

Scheckel, Claudia ; Aguzzi, Adriano

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# 1    **Prions, prionoids, and protein misfolding disorders**

2    *Claudia Scheckel and Adriano Aguzzi*

3

4    Institute of Neuropathology,

5    University of Zurich,

6    Schmelzbergstrasse 12

7    CH-8091 Zurich, Switzerland

8

9    Correspondence to AA

10    e-mail: [adriano.aguzzi@usz.ch](mailto:adriano.aguzzi@usz.ch)

## ABSTRACT

Prion diseases are progressive, incurable and fatal neurodegenerative conditions. The term “prion” was first nominated to express the revolutionary concept that a protein could be infectious. We now know that prions consist of PrP<sup>Sc</sup>, an aggregated form of the cellular protein PrP<sup>C</sup>. Over the years, the term has been semantically broadened to describe aggregates irrespective of their infectivity and the prion concept is now being applied, perhaps overenthusiastically, to all neurodegenerative diseases that involve protein aggregation. Indeed, recent studies suggest that prion diseases (PrDs) and protein misfolding disorders (PMDs) share some common disease mechanisms, which could have implications for potential treatments. Nevertheless, the transmissibility of *bona fide* prions is unique and PrDs should be considered as distinct from other PMDs.

## [H1] INTRODUCTION

**Prion diseases [G]** (PrDs), which are also termed transmissible spongiform encephalopathies (TSEs), are fatal neurodegenerative diseases characterized by neuronal loss, vacuolation, astrocyte activation and microglia activation. PrDs can undergo extraordinarily long incubation periods ranging from years to decades. However, when the clinical signs become evident, the course of the disease is often dramatic<sup>1</sup>.

The term **prion [G]** was originally coined to describe the infectious proteinaceous agent causing PrDs<sup>2</sup>, and did not have a specific biophysical meaning attached to it. Subsequently, prions were shown to consist primarily of PrP<sup>Sc</sup><sup>3</sup>, **aggregates [G]** of the cellular prion protein PrP<sup>C</sup><sup>4</sup>. Misfolded PrP<sup>C</sup> is incorporated into heterodisperse, fibrillary beta-sheet rich structures, which are termed amyloids. Other proteins can also form amyloids, which have been associated with numerous other **protein-misfolding disorders (PMDs) [G]**.

Prions are thought to multiply by a nucleation-and-fragmentation process akin to the growth of crystals<sup>5,6</sup>: highly ordered PrP<sup>Sc</sup> oligomers incorporate endogenous PrP<sup>C</sup>, thereby growing in size (Figure 1). Large PrP<sup>Sc</sup> aggregates may then decay into smaller fragments of various sizes, each of

1 which can restart the nucleation-fragmentation cycle. The minimal self-replicating unit of misfolded  
2 aggregates is called a **propagon** [G]<sup>7</sup>. A propagon reflects the biological activity of the prion rather than  
3 a specific structural entity. Accordingly, a prion sample containing many smaller oligomers has a higher  
4 number of propagons than one containing larger fibrils. Prions show a remarkable resistance to  
5 proteases, heat and decontamination methods, which has proven to be a major challenge for the  
6 prevention of PrDs. Yet, protease resistance of prions only correlates loosely with infectivity: the  
7 majority of infectivity is associated with protease-sensitive oligomers<sup>8</sup>.

8 The process of generating infectious PrP<sup>Sc</sup> has been reproduced in vitro<sup>9</sup>, which has provided substantial  
9 evidence that prion infectivity depends on PrP<sup>Sc</sup>. In vitro amplification of PrP<sup>Sc</sup> allows the detection of  
10 minute amounts of prions and has been adopted for the diagnosis of PrDs (Box 1). Since the  
11 incorporation of PrP<sup>C</sup> is required for prion replication, mice lacking PrP<sup>C</sup> are resistant to prion  
12 infection<sup>4,10,11</sup>. While it is widely accepted that PrP<sup>Sc</sup> is an essential component of the infectious agent,  
13 additional co-factors are likely to play a part in prion replication in vivo<sup>12</sup>. Interestingly, while PrP<sup>C</sup> is  
14 abundantly expressed throughout the body, prion deposition as well as vulnerability to prion toxicity  
15 varies profoundly between tissues<sup>13</sup>. The observation that different cell types show distinct  
16 susceptibilities to prion infection and toxicity further suggests that additional components (proteins or  
17 otherwise) can affect the ability of prions to replicate and/or exert toxicity.

18 An increasing number of neurodegenerative disorders including Alzheimer's Disease (AD),  
19 Parkinson's Disease (PD) and amyotrophic lateral sclerosis (ALS), but also metabolic diseases and  
20 cancer have now been linked to protein misfolding and aggregation<sup>14,15</sup>. While protein aggregation may  
21 conceivably lead to protein inactivation via sequestration, the aggregates themselves can exert toxicity  
22 by interfering with intracellular functions or cell-to-cell signaling. Several protein aggregates linked to  
23 these disorders have been shown, like prions, to undergo cycles of nucleation and fragmentation. Unlike  
24 for prions, no inter-individual transmissibility has yet been demonstrated for any of these aggregates -  
25 we therefore introduced the term "**prionoids**" [G]<sup>14,15</sup> to describe these aggregates. However, shared  
26 characteristics with prions combined with the high prevalence of many prionoid-mediated PMDs have  
27 raised concerns related to the handling of prionoids<sup>15</sup>.

In this Review, we provide an overview of the current understanding of prions and PrDs. We discuss similarities and important distinctions between PrDs and prionoid-mediated PMDs and their respective aggregates, and comment on the implications for the diagnosis, treatment and containment of these diseases. Finally, we highlight different therapeutic strategies that aim to prevent or eliminate pathological protein aggregation and are therefore relevant to PMDs in general.

## [H1] PRION DISEASE

**[H2] Forms of prion diseases.** Human PrDs can be grouped into genetic, sporadic and acquired forms and have an overall incidence of 1-2 cases per million. All PrDs are characterized by an accumulation of PrP<sup>Sc</sup> in the central nervous system, either in the form of plaques or as synaptic deposits. Genetic PrDs (gPrDs) are all caused by mutations in the *PRNP* gene, which encodes PrP<sup>C</sup>, and include genetic Creutzfeldt-Jakob disease (gCJD), Fatal Familial Insomnia (FFI) and Gerstmann-Straeussler-Scheinker Syndrome (GSS) (Figure 2). By contrast, sporadic CJD (sCJD) and sporadic FFI have an unknown etiology despite sCJD being the most common form of human PrDs, accounting for 85-90% of all cases. Acquired PrDs are induced by transmission of pre-existing prions and they include: variant CJD (vCJD), which is caused by bovine prions<sup>16</sup>; iatrogenic CJD (iCJD), which is transmitted by medical procedures; and kuru in Papua New Guinea, which is acquired as a result of cannibalistic rituals. PrDs occur in many mammalian species, most notably as bovine spongiform encephalopathy (BSE or mad cow disease)<sup>17</sup>, scrapie in sheep and goats, and chronic wasting disease in deer and elk<sup>18</sup>. Prions from one species are usually less infectious to individuals of another species, reflecting a species barrier. However, PrDs can also be transmitted between species, albeit with variable efficiency<sup>19</sup>.

**[H2] PrP<sup>C</sup> is required for prion diseases to occur.** Although PrP<sup>C</sup> was first linked to PrDs decades ago, its cellular function is not entirely understood. The 253 amino acid membrane protein (Figure 2a) is evolutionarily conserved from birds to mammals and comprises: a flexible tail at the N-terminus, which spans two charge clusters (CC1 and CC2); an octapeptide repeat region (OR); a hydrophobic domain (HD)<sup>20</sup>; and a C-terminal globular domain, consisting of three  $\alpha$ -helices and two short antiparallel  $\beta$ -sheets<sup>21</sup>. A glycosyl phosphoinositol (GPI) modification at residue 230 anchors PrP<sup>C</sup> to the plasma

membrane. Addition of oligosaccharides at residues 181 and 197 gives rise to different glycosylated forms of PrP<sup>C</sup>, and facilitates the correct localization of PrP<sup>C</sup> to the plasma membrane. Initial functional analysis performed in *Prnp* deficient mice with a mixed genetic background suggested a number of functions for PrP<sup>C</sup>, including a role in regulating long-term potentiation, which underlies memory formation<sup>22</sup>. However, careful replication of experiments in perfectly co-isogenic mice has clarified that some phenotypes, such as enhanced phagocytosis, are due to polymorphisms [G] in genes flanking *Prnp*<sup>23</sup>, including *Sirpa*<sup>24</sup>, which encodes the signal regulatory peptide  $\alpha$ . Nonetheless, all *Prnp* deficient mice develop a chronic demyelinating neuropathy<sup>23,25</sup>, and PrP<sup>C</sup> has been shown to promote myelin homeostasis by activating the G protein-coupled receptor Gpr126 on Schwann cells<sup>26</sup>. The availability of co-isogenic *Prnp* deficient mice now allows a thorough reassessment of functions previously attributed to PrP<sup>C</sup>, and is likely to reveal novel PrP<sup>C</sup> functions.

**[H2] Mutations in PRNP are linked to genetic prion disease.** All known gPrDs are caused by mutations in the *PRNP* gene, which usually have full penetrance [G] (Figure 2b). Most of the mutations are localized in the second and third alpha helix, and are thought to induce PrP<sup>C</sup> misfolding and, ultimately, pathological aggregates via a mechanism that is poorly understood. GSS is characterized by large, multicentric amyloid plaques and the most commonly associated mutation is a proline-to-leucine substitution at codon 102 (P102L)<sup>27</sup>. By contrast, FFI is caused by an asparagine and a methionine at positions 178 (D178N) and 129, respectively<sup>28</sup>. The D178N mutation has additionally been shown to cause gCJD, if in conjunction with a valine at residue 129 (ref<sup>29</sup>). The polymorphism at position 129 has since been observed to affect the aggregation propensity of D178N mutant PrP<sup>C</sup> into amyloid fibrils in vitro, but the underlying mechanism is unknown<sup>30</sup>. Several other point mutations, most notably E200K<sup>31</sup> and V210I<sup>32</sup>, have been associated with gCJD (Figure 2), many of which reside in the globular domain at the C-terminus and disrupt potential salt bridge or hydrogen bonding interactions<sup>33</sup>. In addition, insertions of additional octapeptide repeats cause gCJD and affect the aggregation propensity of PrP<sup>C</sup>.

**[H2] PRNP polymorphisms modulate susceptibility to prion disease.** PrDs are classified as a single

homogenous disease, however it has become evident that prions can cause many different molecular and clinical phenotypes, possibly reflecting the existence of distinct structural assemblies, also termed **prion strains** [G]<sup>34</sup>. Indeed, *PRNP* polymorphisms have been shown to influence the predisposition towards sporadic, variant and genetic PrDs (Figure 2b) in a prion-strain dependent manner. An important disease-modifying polymorphism exists at codon 129, which can encode either valine (V) or methionine(M)<sup>35</sup>. Allele frequencies vary between populations: in the UK, 47% of the normal population are heterozygous at this locus, and 42% and 11% are homozygous for M and V, respectively<sup>36</sup>. Homozygosity for either amino acid predisposes to sCJD and leads to an earlier onset of gPrD<sup>37</sup> and all but one out of >300 vCJD patients identified to date have been homozygous for methionine at codon 129 (ref<sup>38</sup>). Moreover, three of the four individuals that died of vCJD after having received contaminated blood transfusions were homozygous for methionine at codon 129 (ref<sup>39,40</sup>). By contrast, the fourth individual was 129<sup>Met/Val</sup> heterozygous, displayed prion protein deposition only in the spleen and lymph nodes, showed no signs of a neurological disorder, and died of a ruptured abdominal aortic aneurysm<sup>41</sup>. This suggests that while subjects with MV and VV versions of *PRNP* might be able to succumb to vCJD infection, the incubation time in these patients will be significantly longer. Consequently, it has been argued that a large number of individuals may be infected with PrD but remain asymptomatic, and that these individuals might unknowingly transmit the disease during the prolonged incubation periods. While this is theoretically possible, no evidence has come forward to support this idea. Interestingly, MV heterozygosity confers protection against vCJD but not against kuru<sup>42</sup>.

A different polymorphism has been found to affect susceptibility to sCJD in the Japanese population. Codon 219 can encode either glutamic acid or lysine, and 14% of the population has been reported to be heterozygous at this codon (the remaining 86% are homozygous for glutamic acid). However, no heterozygous sCJD patients have been identified to date, which suggests that heterozygosity at codon 219 may protect individuals from developing sCJD<sup>43</sup>. More recently, another protective polymorphism, this time at codon 127, has been described specifically in populations from kuru-exposed regions. Interestingly, heterozygosity for glycine and valine was observed in non-diseased individuals but not

1 in patients with kuru, who were all homozygous for valine<sup>42</sup>. The G127V variant was further assessed  
2 in mice, revealing that it conferred protection not only against kuru but also against classical CJD prion  
3 strains<sup>44</sup>.

4  
5 **[H2] Non-PRNP genetic susceptibility factors.** The low incidence of PrDs renders the discovery of  
6 genetic modulators of PrD a major challenge, and genome-wide association studies revealed only *PRNP*  
7 to being highly associated with a risk for all human PrDs<sup>45-47</sup>. A recent study aimed to quantify PrD  
8 penetrance by leveraging previously published datasets. The authors collected sequencing data from  
9 ~16,000 PrD cases from around the world, constituting a substantial fraction of all documented PrD  
10 cases to date. The PrD cases were then compared to a control group consisting of ~61,000 exomes from  
11 unrelated individuals and GWAS data from ~530,000 customers of the genetic analysis company,  
12 23andMe. Remarkably, in these large control population cohorts, 63 rare *PRNP* genetic variants  
13 previously reported to cause PrD were observed 30 times more often than expected based on the  
14 incidence of gPrDs. The overrepresentation of *PRNP* mutations was not limited to specific ethnic or  
15 demographic groups but was observed in populations of diverse ancestries<sup>48</sup>. While several of these  
16 variants might in fact represent benign or low-risk variants, this data nonetheless suggests the existence  
17 of non-genetic factors that affect disease manifestation. Environmental factors have been found to affect  
18 the pathogenesis of most diseases characterized to date and are therefore certain to also contribute to  
19 PrDs. It is also possible that some healthy subjects with *PRNP* mutations have developed mechanisms  
20 that protect them from developing disease. One tantalizing hypothesis is that these individuals produce  
21 PrP<sup>Sc</sup>-specific antibodies that shield them against the pathogenic effects of *PRNP* mutations.

## 23 **[H1] PRIONOID-MEDIATED DISORDERS**

24 The term ‘prion’ has been liberally used for many protein aggregates. Yet *bona fide* infectivity of these  
25 aggregates, exemplified by serial transmissibility through consecutive hosts to prove unlimited self-  
26 replication of the agent, has been rarely claimed. Mammalian protein aggregates that are defined as  
27 prions will need to be handled in accordance with high level biosafety measures, which may include



1 the requirement for BSL3 laboratories. As we believe that the necessity of such measures should be  
2 determined by data rather than imprecise semantics, we have proposed that the term ‘prionoid’ should  
3 be used to describe misfolded protein aggregates for which transmissibility between individuals has not  
4 yet been demonstrated<sup>14,15</sup>.

5  
6 **[H2] Non-neuronal prionoids.** The transcriptional regulator p53 has long been known to be a tumor  
7 suppressor, and p53 mutations have been detected in >50% of human malignant tumors. More recently,  
8 mutated p53 has been shown to form aggregates in tumors and cancer cell lines<sup>49,50</sup>. Mutations in the  
9 p53 DNA binding domain destabilize its tertiary structure, leading to the exposure of an aggregation-  
10 nucleating segment (also termed amyloid adhesive segment) that is normally buried within the  
11 hydrophobic core of the protein. The exposure of this fragment is then thought to trigger the aggregation  
12 of wild type p53 and its paralogs, p63 and p73, into beta-sheet-like structures, which form large  
13 cytoplasmic inclusions<sup>49</sup>. Additionally, p53 aggregates have been shown to spread between cells in a  
14 manner that is similar to cell-to-cell transmission of prions<sup>51,52</sup>. Indeed, mice and patients harboring p53  
15 aggregation mutations have higher tumor numbers than those with non-aggregation mutations, and  
16 tumor formation is dependent on the presence of aggregation-prone p53<sup>53</sup>. These findings demonstrate  
17 that p53 is a *bone fide* prionoid, and indicate that p53 aggregation and cell-to-cell transmission play an  
18 important role in metastasis formation.

19 Misfolded aggregates of islet amyloid polypeptide (IAPP) accumulate in the pancreas and are  
20 commonly observed in type 2 diabetes (T2D) patients. IAPP aggregates that have either been generated  
21 in vitro or obtained from pancreatic samples induce the misfolding and deposition of endogenous IAPP  
22 in mice, confirming IAPP to be a prionoid. Importantly, IAPP deposition is accompanied by typical  
23 T2D traits such as hyperglycemia, impaired glucose tolerance and a decrease in pancreatic  $\beta$ -cells,  
24 indicating that IAPP accumulation plays an important role in T2D manifestation<sup>54</sup>.

25 Although protein aggregation usually occurs in specific organs, several PMDs are characterized by  
26 systemic aggregate deposition. Examples include aggregation of immunoglobulin light chain in  
27 amyloid light (AL) amyloidosis, transthyretin in familial amyloid polyneuropathy,  $\beta$ 2-microglobulin in

dialysis-related amyloidosis and amyloid A (AA) in reactive amyloid A amyloidosis. It has been suggested that transmission of these aggregates can occur between multiple species, indicating that they might not only qualify as prionoids but even as prions, with the evidence being strongest for AA <sup>55</sup>.

**[H2] Prionoids linked to neurodegeneration.** Aggregates of  $\alpha$ -synuclein have been linked to multiple neurodegenerative diseases, including multiple system atrophy (MSA), dementia with Lewy bodies (DLB) and PD. Intracerebral inoculation of mice with brain homogenate from MSA patients induces  $\alpha$ -synuclein phosphorylation and aggregation and neurological phenotypes, even upon serial propagation<sup>56,57</sup>. The detection of infectivity after multiple passages is a hallmark of prions, suggesting that  $\alpha$ -synuclein might indeed be a prion. However, the development of CNS dysfunction in these mice is dependent on the presence of a hemizygous transgene encoding an aggregation-prone mutant form of human  $\alpha$ -synuclein, which by itself did not cause neurological dysfunction. This raises questions as to whether only mutated  $\alpha$ -synuclein can be incorporated into  $\alpha$ -synuclein aggregates, or whether the requirement for a transgene reflects an inter-species barrier similar to that observed for known prions <sup>58</sup>. Interestingly, neither PD nor DLB patient homogenates could induce neurological dysfunction in mice hemizygous for the mutated human  $\alpha$ -synuclein transgene. This result indicates that the  $\alpha$ -synuclein aggregates from different diseases not only vary in the cell type in which they are found (neuronal inclusions in DLB and PD; glial inclusions in MSA), but also in their potential to be propagated in mice. MSA-associated  $\alpha$ -synuclein inclusions in glia cells might be more infectious due to their glial origin, however MSA homogenates can also induce  $\alpha$ -synuclein aggregation in neurons<sup>57</sup>. A recent study showed that even spinal cord homogenates prepared from wild-type and  $\alpha$ -synuclein deficient mice could induce  $\alpha$ -synuclein deposits and CNS dysfunction in mice hemizygous for the mutated human  $\alpha$ -synuclein transgene<sup>59</sup>. This observation suggests that these homogenates contain a component that can trigger  $\alpha$ -synuclein pathology in the presence of mutated human  $\alpha$ -synuclein. Perhaps even more worrisome are reports on human PD patients that received embryonic neuronal transplants. Despite the young age of the transplanted neurons, the grafts displayed synuclein inclusions

1 10-24 years post-transplantation, showing that synuclein aggregates were able to spread from host to  
2 graft in a prion-like manner<sup>60-64</sup>.

3 AD is characterized by amyloid- $\beta$  ( $A\beta$ ) deposition, which has been shown to follow a stereotypic  
4 sequence that involves progressively larger brain areas<sup>65</sup>. The injection of human AD brain  
5 homogenates containing  $A\beta$  aggregates causes cerebral  $\beta$ -amyloidosis and pathology in mice.  
6 Importantly,  $A\beta$ -immunodepleted homogenates failed to induce lesions, suggesting that induction of  
7 amyloidosis is dependent on  $A\beta$ <sup>66</sup>. However, similar to synuclein, the induction of  $A\beta$  pathology in  
8 mice depends on the overexpression of  $A\beta$ . Further experiments demonstrated that  $A\beta$  alone is indeed  
9 sufficient for self-propagation in vitro and that the in vitro generated  $A\beta$  aggregates are able to induce  
10 amyloidosis<sup>67</sup>. Interestingly, it has recently been shown that eight deceased individuals that contracted  
11 iCJD via human growth hormone (hGH) injections also display  $A\beta$  pathology<sup>68</sup>. These findings were  
12 confirmed in a separate study, which additionally detected  $A\beta$  accumulation in 12 hGH recipients that  
13 died of a cause other than CJD<sup>69</sup>. It remains unclear if the hGH samples were the source of  $A\beta$   
14 aggregation, and the patients did not show any signs of tau pathology, a second hallmark of AD.  
15 Nonetheless, these subjects might represent the first known cases of iatrogenic  $A\beta$  transmission.  
16 Consistently, iCJD caused by dural grafting has been shown to be associated with  $A\beta$  pathology<sup>70</sup>,  
17 making  $A\beta$  a candidate prion.

18 Tau pathology is not only characteristic of AD but also of multiple other neurodegenerative disorders.  
19 Similarly to other prionoids, Tau aggregates spread throughout the brain in an orderly fashion that is  
20 characteristic for each tauopathy, ultimately leading to distinct Tau pathologies<sup>71</sup>. However, the cause  
21 of Tau aggregation in the different diseases is still mostly unknown. The injection of AD homogenates  
22 containing Tau aggregates into mice has been shown to induce Tau aggregation, even in wildtype  
23 mice<sup>72</sup>. Furthermore, a recent study revealed that some hGH-related iCJD patients display tau pathology  
24 that seems to be linked to Tau contaminants in the respective hGH samples<sup>73</sup>. While these results might  
25 have far-reaching implications for the handling of tauopathy patients and samples, further validation is  
26 required to confirm the transmission of Tau aggregates between individuals before it can be considered

1 a prion.

## 3 [H1] MEDIATORS AND MODULATORS OF TOXICITY

4 **[H2] Mechanisms underlying aggregation differ between protein misfolding disorders.** The formation  
5 of extracellular and intracellular protein aggregates can exert toxicity both in the extracellular space and  
6 within the cell. Furthermore, aggregation goes hand in hand with the sequestration of monomeric  
7 protein, which can cause additional deleterious effects. For example, the deleterious effects of p53  
8 aggregation in cancer seem to be associated with sequestration of p53 rather than with the aggregates<sup>49</sup>.  
9 By contrast, the toxicity of PMDs affecting the nervous system seems to be exerted by the aggregates  
10 themselves. Some of the aggregated proteins possess a low-complexity domain that is intrinsically  
11 disordered and enriched for polar uncharged residues, particularly glutamine and asparagine<sup>74</sup>. Such  
12 domains have been called “prion-like” owing to their similarity to certain nucleating proteins of yeast.  
13 However, PrP<sup>C</sup> lacks such a domain, indicating that the early aggregation events leading to the  
14 formation of prions and prionoids are distinct. Indeed, certain proteins involved in neurodegeneration  
15 undergo **phase demixing [G]**<sup>75</sup>, a recently discovered aggregation modality that is often reversible and  
16 fundamentally different from the aggregation of prions.

17 While the cause, the location and the aggregates themselves differ, certain parallels between the  
18 different PMDs can be legitimately drawn. For instance, oligomers of misfolded proteins are more  
19 pathogenic than higher-order structures such as protofilaments and fibrils<sup>8</sup>, possibly because of their  
20 higher stoichiometry. Furthermore, disparate aggregates often trigger converging pathways of toxicity.  
21 Hence insights gained for one PMD may be relevant for others.

22 **[H2] Uncoupling protein aggregation and toxicity.** Neurons devoid of PrP<sup>C</sup> do not develop spongiform  
23 changes even when chronically exposed to prions *in vivo*<sup>11</sup>, suggesting that PrP<sup>C</sup> is not only needed for  
24 prion replication, but that it also functions as a mediator of prion toxicity. Additionally, this observation  
25 indicates that extracellular PrP deposits are not toxic *per se* but that binding of aggregates to membrane-  
26 bound PrP<sup>C</sup> is required to induce toxicity within cells. This hypothesis is further supported by the  
27 observation that protective PrP<sup>C</sup>-directed antibodies prevent neurotoxicity without affecting prion

1 accumulation<sup>76</sup>. Antibodies against PrP<sup>C</sup> were shown to be protective against PrD in mice almost two  
2 decades ago<sup>77</sup> and a series of monoclonal antibodies targeting different domains of PrP<sup>C</sup> has since  
3 proven useful for studying the mechanism of prion-induced toxicity<sup>78</sup>. Protective antibodies binding to  
4 the flexible tail (FT) of PrP<sup>C</sup>, as well as FT deletion mutants of PrP<sup>C</sup>, revealed that FT is required for  
5 prion replication *in vivo* and is the effector domain of PrP<sup>Sc</sup>-mediated toxicity<sup>76</sup>. By contrast, antibodies  
6 targeting the globular domain (GD) of PrP<sup>C</sup> induce transcriptional changes and phenotypic changes  
7 remarkably similar to those induced by prions, including neuronal loss, astrogliosis, microglial  
8 activation, and spongiosis<sup>79</sup>. Anti-GD antibodies and prions also activate similar toxicity pathways *ex*  
9 *vivo*<sup>76,79,80</sup>. However, anti-GD antibodies fail to induce aggregates, infectious prions and prion  
10 pathology *in vivo*<sup>81</sup>, indicating that they act on a pathway downstream of prion replication. The fact that  
11 PrP<sup>C</sup> antibodies can be protective or toxic, depending on the targeted domain, has shed light on the  
12 mechanisms of prion-induced toxicity and has far-reaching implications for immunotherapy of not only  
13 PrDs but diseases more generally.

14 **[H2] Aggregation-induced toxicity.** The observation that PrP<sup>C</sup> is required for toxicity suggests that  
15 precluding protein aggregates from entering the cell might prevent the induction of toxicity and  
16 neuronal loss. Several mechanisms have been proposed to explain how aggregates enter and spread  
17 between cells, which involve exosomes<sup>82,83</sup>, nanotubes<sup>84</sup> or receptor-mediated internalization<sup>8</sup>.  
18 However, the mechanisms by which extracellular aggregates initiate intracellular toxicity are less clear.  
19 One possibility is that aggregates alter receptor-mediated signaling pathways. Aberrant glutamate  
20 signaling has been linked to PrD<sup>85-88</sup>, which is further supported by the observation that PrP<sup>C</sup> inhibits  
21 N-methyl-D-aspartate receptors (NMDAR) and attenuates **excitotoxicity** **[G]**<sup>89</sup>. Aβ oligomers inhibit  
22 long-term potentiation (LTP) and impair synaptic plasticity, and several receptors have been suggested  
23 to play a role in internalizing Aβ, including PrP<sup>C</sup>. Indeed, it has been claimed that PrP<sup>C</sup> is required for  
24 Aβ-induced LTP in hippocampal slices<sup>90</sup> and for memory impairment *in vivo*<sup>91</sup>. mGluR5 may act as a  
25 co-receptor for Aβ binding to PrP<sup>C</sup><sup>92,93</sup>, with subsequent NMDAR activation leading to synaptic spine  
26 loss<sup>94,95</sup>. Accordingly, increased glutamate signaling has been seen in a mouse AD model<sup>96,97</sup> and  
27 genetic depletion of mGluR5 reduces AD pathology *in vivo*<sup>98</sup>. However, the role of PrP<sup>C</sup> in mediating

1 A $\beta$  toxicity is contentious. Several studies suggest that A $\beta$  oligomers can induce synaptic defects and  
2 impair long-term memory formation independently of PrP<sup>C</sup> <sup>99,100</sup> and neither PrP<sup>C</sup> ablation nor  
3 overexpression modified the synaptic pathology in two mouse AD models<sup>101,102</sup>. These discrepancies  
4 can likely be explained by differences in study design, including the use of different mouse AD models,  
5 and will almost certainly be resolved by future studies. More recently, it has been suggested that PrP<sup>C</sup>  
6 also mediates the uptake of  $\alpha$ -synuclein oligomers<sup>103,104</sup>. Oligomeric  $\alpha$ -synuclein is highly neurotoxic  
7 and impairs hippocampal LTP via NMDAR activation<sup>105</sup>. The interaction of  $\alpha$ -synuclein and PrP<sup>C</sup> at  
8 the post-synapse activates NMDAR via mGluR5, and triggers synaptic defects and cognitive  
9 impairment<sup>104</sup>.

10 By contrast, intracellular aggregates might mediate toxicity by affecting subcellular compartments, such  
11 as the endoplasmic reticulum (ER). PrP<sup>C</sup> undergoes posttranslational modifications in the ER and Golgi  
12 before localizing to cholesterol-rich lipid rafts at the plasma membrane. PrP<sup>C</sup> has a short half-life<sup>106</sup>,  
13 and approximately 10% is misfolded and subsequently degraded by the ubiquitin-proteasome system  
14 (UPS) after retrograde ER translocation<sup>107,108</sup>. By contrast, pathogenic mutations linked to gPrD cause  
15 PrP<sup>C</sup> to aggregate and remain in the ER and Golgi<sup>109-113</sup>. Dysfunctional and misfolded proteins are  
16 usually ubiquitinated and degraded by the UPS, and it has been suggested that this process is inhibited  
17 by misfolded PrP<sup>114-116</sup>. The resulting buildup of dysfunctional proteins eventually causes ER stress and  
18 activates the unfolded protein response (UPR). One consequence of UPR induction is a global shutdown  
19 of translation, mediated by phosphorylation of PERK, which in turn phosphorylates and deactivates the  
20 eukaryotic translation initiation factor eIF2 $\alpha$ . Prion infection causes a global repression of protein  
21 synthesis via eIF2 $\alpha$  phosphorylation, ultimately leading to synaptic dysfunction and neuronal loss.  
22 Interestingly, globally increasing translation via eIF2 $\alpha$  dephosphorylation reduces neuronal toxicity and  
23 increases the survival time of prion-exposed mice, whereas increasing eIF2 $\alpha$  phosphorylation further  
24 aggravates prion-induced pathology<sup>117</sup>. ER stress, as well as activation of the UPR and PERK, have  
25 also been reported in several other PMDs including AD, PD, ALS, and tauopathy<sup>118-123</sup>.

26  
27 **[H2] Differential vulnerability of cells and tissues.** Protein aggregation has been linked to several non-  
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neuronal disorders, including metabolic diseases and cancer, but the brain is the organ most vulnerable to protein aggregation. While PMD aggregates are thought to directly exert toxicity on the brain, several of their substrates, including PrP<sup>C</sup>, amyloid precursor protein,  $\alpha$ -synuclein and tau, are not exclusively expressed in the nervous system<sup>124-126</sup>. PrP<sup>C</sup> is expressed at moderate levels in heart, muscle and spleen, and prions have been shown to accumulate in these tissues. In fact, prions replicate in peripheral lymphoid organs before they reach the brain, and mice that lack B lymphocytes, or follicular dendritic cells (FDCs), cannot succumb to PrD if infected by peripheral administration<sup>127</sup>. These studies demonstrate that prion infectivity and pathogenesis are not restricted to the brain, but that prion-diseased mice, as well as patients, are likely to succumb to fatal neuronal defects before non-neuronal phenotypes can manifest. On the other hand, certain organs never acquire prion replication competence, even when forced by transgenesis to express high levels of PrP<sup>C</sup><sup>128</sup>. It is likely that a combination of substrate expression and exceptional vulnerability of neurons account for the predominant neuronal phenotype of not only PrD, but also other neurodegenerative PMDs including AD, PD, and ALS.

Each neurodegenerative disease displays a distinct pathology within the central nervous system, which is determined by a variety of factors, including differences in the aggregate structure and localization, and selective vulnerabilities of cells and brain regions (Box 2). For example,  $\alpha$ -synuclein aggregates are present as cytoplasmic inclusions in multiple neurodegenerative diseases including PD, DLB and MSA, and yet the affected cell types and brain regions vary substantially between the different diseases. The underlying cause for the pathological differences in  $\alpha$ -synuclein deposition is unknown but, not surprisingly, they result in distinct clinical manifestations<sup>129</sup>. While pathological changes are usually homogenous within one neurodegenerative disease, PrDs are characterized by a spectrum of different pathologies and clinical features. One cell type that is particularly vulnerable to prion deposits and other protein aggregates is parvalbumin positive inhibitory neurons, which are distinguished by a high firing rate and a high metabolic rate, leading to increased exposure to oxygen radicals and intracellular damage. Severe selective loss of parvalbumin neurons in the cortex and hippocampus has been observed in CJD, GSS and kuru<sup>130-132</sup>. By contrast, FFI pathology is mostly focused on the thalamus and patients show only a moderate loss of cortical parvalbumin neurons<sup>132</sup>. The differential vulnerability of cells and

1 brain regions indicates that aggregation-induced toxicity can be modulated by the expression of co-  
2 factors, such as receptors and chaperones<sup>12</sup>. Future studies focusing on single cells, or at least single  
3 cell types, will therefore be of particular importance in deciphering why various cell types undergo  
4 distinct fates upon exposure to protein aggregates. The remarkable phenotypic heterogeneity of PrDs is  
5 further attributed to the different biochemical and neuropathological profiles of the various prion  
6 strains, which seem to be able to exert differential toxicity, presumably through their interplay with  
7 additional factors<sup>12</sup>.

8 Immune cells are critical for prion replication and spreading, especially when prions are administered  
9 via the peripheral route. Similarly to PrP prions, the induction of  $\alpha$ -synuclein pathology seems to be  
10 strongly dependent on the source of the injected homogenate and the route of administration. Mice  
11 inoculated intracerebrally with homogenates containing  $\alpha$ -synuclein aggregates that have been taken  
12 from MSA patients display a more rapid disease progression compared with intraperitoneally inoculated  
13 mice<sup>133</sup>. A $\beta$  aggregates were recently shown to be able to enter the brain via the blood stream using a  
14 **parabiosis [G]** model in which wildtype mice showed hippocampal impairment upon being paired with  
15 transgenic AD mice<sup>134</sup>. This observation is in contrast to prions, which do not enter the nervous system  
16 via the blood stream but via peripheral, mostly sympathetic, nerves<sup>135,136</sup>.

17 Post-translational modifications have been suggested to impact protein aggregation, replication and  
18 toxicity. Different PrDs have been linked to distinct ratios of mono- and di-glycosylated PrP<sup>C</sup><sup>137</sup>, and  
19 several post-translational modifications have also been implicated in AD pathogenesis. For instance,  
20 accumulation of N-terminally truncated and pyroglutamated A $\beta$  precedes the deposition of non-  
21 modified A $\beta$ <sup>138</sup>, and inhibition of glutamyl-cyclase, which generates pyroglutamated A $\beta$ , improves  
22 neuronal defects and attenuates AD pathology in mice<sup>139</sup>. Nitrosative stress has also been shown to be  
23 induced in AD, which leads to NOS2 mediated addition of 3-nitrotyrosine to proteins, including  
24 A $\beta$ <sup>140,141</sup>. Nitrated A $\beta$  can then induce and accelerate amyloidosis and exacerbate memory loss, both of  
25 which can be prevented by NOS2 inhibition. Hence targeting disease-specific posttranslational  
26 modifications of aggregates might represent a promising approach to combat PMDs.

27 **[H2] Chaperones.** Chaperones linked to protein synthesis (CLIPs) stabilize and correctly fold newly



1 synthesized proteins, whereas heat shock proteins (HSPs) recognize misfolded proteins (Figure 1).  
2 CLIPs are downregulated and HSPs are induced in cells of the aging brain or in response to stress,  
3 which protects the cells against misfolded protein toxicity. Stress, aging and mutations can induce  
4 protein misfolding and expose otherwise buried aggregation-prone domains. If not targeted by  
5 chaperones for either re-folding or proteasomal degradation, these misfolded proteins start to aggregate  
6 into higher-order structures that are resistant to proteasomal degradation<sup>142</sup>. Notably, different HSPs  
7 can have opposing effects on protein aggregation. For example, whereas HSP70 promotes protein  
8 degradation via the ubiquitin proteasome system, HSP90 stabilizes proteins and inhibits their  
9 ubiquitination. The activity of these two proteins is regulated in a coordinated manner, with inhibition  
10 of HSP90 leading to HSP70 activation via HSF1. Compared with wildtype mice, mice lacking  
11 functional HSF1 have a shortened lifespan when inoculated with prions but the resulting behavioral and  
12 pathological changes are similar<sup>143</sup>, which suggests that HSF1 exerts its protective function only after  
13 the onset of clinical symptoms. The co-ordinated regulation of HSP70 and HSP90 makes these  
14 chaperones interesting therapeutic targets. Indeed, HSP90 inhibitors have been shown to prevent  
15 aggregation and toxicity of many aggregates in cells and mice, including p53,  $\alpha$ -synuclein, tau and  
16 huntingtin<sup>53,144-147</sup>, probably reflecting consequences of HSP90 inhibition as well as HSP70 activation.  
17 Several chaperones have been shown to be upregulated in PrD patients, including GRP78 and GRP58  
18 (ref<sup>148,149</sup>). GRP78 (also known as BiP or HSP5A) prevents the aggregation of misfolded proteins in  
19 the ER, including PrP and A $\beta$ , and targets protein aggregates for proteasomal degradation<sup>112,150</sup>.  
20 Accordingly, GRP78 overexpression reduces prion replication, and GRP78 reduction leads to increased  
21 prion replication and accelerated PrD progression<sup>151</sup>. Similar results have been reported for GRP58  
22 (also termed ERp57) with its overexpression conferring neuroprotection and its downregulation leading  
23 to increased prion induced toxicity<sup>152</sup>.  
24 Another chaperone of interest with respect to protein aggregation is the yeast chaperone Hsp104, a prion  
25 disaggregase that acts together with Hsp70 and Hsp40 to release correctly folded proteins from  
26 aggregates<sup>153</sup>. Yeast prions are highly sensitive to the levels of Hsp104; low levels of Hsp104 promote  
27 oligomer formation, oligomerization is prevented at high Hsp104 concentrations<sup>154</sup>, and loss of Hsp104

eliminates prions<sup>155</sup>. To date, a homologous disaggregase has not been identified in metazoans.

## **[H1] TREATING PROTEIN MISFOLDING DISORDERS**

Several different compounds have been used to fight protein aggregation disorders (Figure 3). While some reagents are designed to interfere with the aggregation process, others eliminate or even hyperstabilize the aggregates. Here we outline some approaches relevant to the treatment of PrDs and other protein aggregation disorders. A more comprehensive discussion of therapeutic principles can be found in a recent review<sup>156</sup>.

**[H2] Inhibition of protein aggregation.** The findings that p53 is a prionoid and that p53 aggregation plays a crucial role in some cancers<sup>157</sup> opens up the potential for novel therapeutic strategies that specifically interfere with p53 aggregation (Figure 3A). The chaperone complex HSP90/HDAC6 has been shown to be upregulated in cancer cells and to stabilize p53 aggregates<sup>158</sup>. Interestingly, currently approved HSP90 inhibitors reduced tumor growth and extended survival time in mice expressing an aggregation-prone version of p53, while mice deficient for p53 were unaffected by HSP90 inhibitor treatment<sup>53</sup>. Furthermore, a peptide that binds the amyloid adhesive segment of p53 prevents p53 aggregation, restores p53 function, and induces cell death and tumor regression in mice<sup>159</sup>. The course of cancer treatment should thus not only be dependent on the identity of a mutated gene, but should also take the type of mutation into account. HSP90 inhibitors have also been applied to mouse models of neuronal PMDs. While effective in preventing  $\alpha$ -synuclein, A $\beta$ , tau and Huntingtin aggregation and toxicity, long-term relief of disease symptoms has proven to be challenging<sup>144-147,160,161</sup>. Recently, HSP90 inhibition was observed to provide synaptic protection in a mouse AD model<sup>160</sup>, suggesting that chaperone modulation might indeed be a promising therapeutic approach for neuronal PMDs in the future.

**[H2] Depletion of substrates with anti-prions.** An orthogonal approach to interfere with protein aggregation is the design of anti-prions. Anti-prions are innocuous PrP aggregates that, upon injection, can compete with prions for the same substrate, PrP<sup>C</sup>, thereby reducing prion replication (Figure 3B).

1 Anti-prions delay the onset of clinical symptoms in prion-injected hamsters and prevent disease  
2 manifestation in animals exposed to low quantities of prions. Interestingly, a single dose of anti-prion  
3 reduced prion infectivity by 99%, making anti-prions an interesting candidate for therapy<sup>162</sup>. Most  
4 therapeutics are rapidly metabolized, consumed or degraded and therefore need to be administered on  
5 a regular basis. By contrast, anti-prions self-replicate and are therefore self-sustaining until their source,  
6 PrP<sup>C</sup>, is depleted. Anti-prions are therefore also tantalizing therapeutics for other neurodegenerative  
7 PMDs. While it is possible that the depletion of the substrate might cause deleterious effects, these may  
8 be limited and tolerable. For instance, mice lacking PrP<sup>C</sup> suffer from relatively mild phenotypes<sup>23</sup>, and  
9 mice without  $\alpha$ -synuclein display no gross morphological or behavioral abnormalities<sup>163</sup>. However,  
10 mice lacking A $\beta$  showed impaired neuronal function<sup>164</sup>. Thus, the pros and cons of substrate depletion  
11 versus inhibition of aggregation will be different for each PMD and will require careful consideration.

12  
13 **[H2] Stabilization of protein aggregates.** The most important stage in the replicative cycle of a prion  
14 is arguably the fragmentation of an aggregate into two or more propagons, as this is the process by  
15 which prions multiply. Indeed, theoretical models have predicted, and studies in experimental models  
16 have validated, that the frangibility of amyloid fibrils is the most important parameter governing the  
17 rate of replication of prions<sup>6</sup>. Thus, any therapeutic strategy based on ‘beta sheet breakers’<sup>165</sup>, which are  
18 homologous peptides that are unable to adopt higher-order structures, might increase rather than reduce  
19 the number of prions. A promising alternative approach is to instead hyperstabilize aggregates to  
20 prevent their fragmentation and replication (Figure 3C). Luminescent conjugated polythiophenes  
21 (LCPs) bind to a variety of amyloids<sup>166</sup>, and have also been shown to bind and stabilize prions<sup>167</sup>. LCPs  
22 optimized for prion binding were efficacious against multiple prions strains<sup>168</sup> and could extend the  
23 lifespan of prion-exposed mice by up to 80%<sup>169</sup>. LCPs are well tolerated in mice and can cross the  
24 blood-brain-barrier, which, together with their high affinity for many amyloids, make them interesting  
25 candidates for therapeutic development.

26  
27 **[H2] Antibodies.** Antibodies are currently considered to be promising therapeutics for the treatment of

1 protein aggregation disorders. By specifically targeting complex and often conformation-dependent  
2 antigens, antibodies are thought to have fewer off-target effects than traditional small-molecule  
3 therapeutics. An increasing number of human-derived antibodies are entering clinical trials for various  
4 diseases, and are thought to have a better safety profile than their “humanized” counterparts<sup>170</sup>.

5 In the case of protein aggregation disorders, it is conceivable that antibodies exert protective effects  
6 through multiple different mechanisms (Figure 3D). For instance, antibodies can bind to monomeric or  
7 aggregated proteins thereby making the substrate unavailable for conversion into aggregates<sup>171</sup> and/or  
8 sterically interfering with the aggregation process itself. Certain prion antibodies have been suggested  
9 to act by a different mechanism, which involves targeting a part of the prion protein that is required for  
10 exerting toxicity; in this case, antibodies engaging the flexible tail can counteract prion-induced  
11 toxicity. While the antibody does not reduce infectivity, it interferes with downstream events triggered  
12 by prions and prevents them from inducing neurodegeneration<sup>79</sup>. Finally, antibodies can specifically  
13 bind to and neutralize aggregates, resulting in more efficient clearance of toxic species from affected  
14 tissues, for instance by phagocytic cells<sup>172-174</sup>. Indeed, research into AD therapeutics focuses on  
15 antibodies that specifically detect and eliminate amyloid deposits. One promising AD drug currently  
16 undergoing clinical trials, aducanumab, is an antibody isolated from a human centenarian who showed  
17 no signs of cognitive impairment. It was speculated that this donor individual may have developed  
18 antibodies against aggregated A $\beta$ , which safeguarded against dementia. Aducanumab targets and  
19 reduces aggregated A $\beta$  in a dose- and time-dependent manner similarly to previously investigated  
20 antibodies but, in contrast to other antibodies, it appears to slow the rate of clinical decline<sup>175</sup>. However,  
21 this study must be viewed in context: there is currently no population-based evidence that spontaneous  
22 immunity against A $\beta$  exists in humans and is protective against AD. Furthermore, as stated above,  
23 certain PrP antibodies are toxic, suggesting that caution should be exercised in clinical trials of  
24 immunotherapies. Nonetheless, in our view, the prospects of immunotherapy for the treatment of  
25 neurodegenerative diseases remain promising.

26 It is also important to note that a reduction of A $\beta$  aggregates does not necessarily impact the clinical  
27 progression of AD, as powerfully demonstrated by the failure of numerous clinical trials despite

convincing pharmacodynamics<sup>176</sup>. Furthermore, the deposition of A $\beta$  aggregates has been observed in individuals without dementia<sup>177</sup> and is thought to occur decades before the onset of clinical symptoms of AD, which seems to correlate with neurodegeneration rather than amyloid deposition<sup>178</sup>. One likely explanation for the failure of many AD trials might therefore lie within the trial design and not the efficacy of the tested compound. Many patients display clinical symptoms at the time of enrolment, a stage where amyloid deposits might have already induced irreversible toxicity. With the development of novel technologies and the identification of new biomarkers, the early diagnosis and enrolment of preclinical AD patients has become possible, and will hopefully yield promising outcomes for upcoming clinical trials. The difficulty of correctly diagnosing AD, combined with the high prevalence of the disease (more than 9% of individuals older than 65), raises the question whether AD therapeutics should be administered prophylactically in the future.

## **[H1] CONCLUSIONS AND FUTURE PERSPECTIVES**

To date, fortunately neither AD nor PD nor any other protein aggregation disease are known to have caused a human epidemic such as kuru and vCJD. However, the prevalence of these diseases is high and their causes are still largely unknown, which complicates the detection of infectious aggregates that can spread between individuals. Nevertheless, from a medical perspective, PrDs currently still stand out as infectious diseases with many similarities to viral encephalopathies; they are therefore profoundly distinct from all other neurodegenerative diseases despite their similarities in molecular pathogenesis. Using its original definition as an infectious protein-, no protein aggregate other than PrP<sup>Sc</sup> can currently be called a prion. It is possible that some of the proteins that currently qualify as prionoids - in particular, the aggregated forms of synuclein<sup>179</sup> and AA amyloid<sup>180</sup> - may have to be reclassified as true prions if they are shown to be infectious.

Several orthogonal therapeutic strategies have been undertaken to combat protein aggregation and its corresponding diseases. Many of these therapies have shown promise *in vitro* and in mice, but it remains to be determined if these results hold up in human studies. Indeed, clinical trials for protein aggregation disorders have, with few exceptions, yielded vastly disappointing results<sup>176</sup>. However, our knowledge

of these diseases has increased tremendously over the past decades. Research on PrDs has historically led the field of PMDs and the mouse model for PrD has been shown to recapitulate transcriptome-wide changes in human patients more faithfully than other neurodegenerative disease models<sup>181</sup>. Studies on prions are therefore likely to continue to drive our understanding of aggregation-induced toxicity. Several similarities between different protein aggregates have been identified, and different aggregates seem to exert toxicity, at least partly, by the same pathways. All of which suggests that insights gained on one of these disorders may be valid for other protein aggregation disorders. These findings, combined with the development of novel technologies, may allow the development of effective treatments for PMDs in the future.

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#### **Author contributions**

Both authors contributed to researching, discussing, writing and editing this Review.

#### **Competing interests**

Adriano Aguzzi is a founder and director of Mabyron Inc., a company devoted to the development of human antibodies for treating intractable diseases, including neurodegeneration. The authors are not aware of any other affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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#### **Reviewer information**

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#### **DISPLAY ITEMS**

##### **Box 1 | Diagnosing prion diseases**

The transmissible nature of prion diseases (PrDs) and the resulting danger of contracting the disease iatrogenically make their correct diagnosis a pressing need for patients, their families and society. Historically, PrDs have been diagnosed based on their clinical symptoms and by excluding other diseases. Diagnosis is further supported by magnetic resonance imaging (MRI), electroencephalography (EEG), and the detection of surrogate markers in the cerebrospinal fluid (CSF). While diffusion patterns on MRI, periodic sharp and slow wave complexes on EEG and an upregulation of CSF markers such as 14-3-3 correlate with PrD, these assays usually simply indicate neuronal damage and even a combination of them is vastly insufficient for providing the sensitive and specific

1 results required<sup>182</sup>.

2 A definitive diagnosis of PrD requires the detection of protease-resistant prion deposits. However, prion  
3 deposits are most prominent in the brain and their ante-mortem detection without a brain biopsy has  
4 proven to be a major challenge. Conventional immunoblotting and ELISA techniques are usually not  
5 sensitive enough to detect the minute amounts of prions in more accessible patient samples, such as  
6 blood. The detection of misfolded PrP is further complicated by the excess of normal PrP<sup>C</sup> in blood,  
7 and even the development of an ultrasensitive ELISA involving enrichment of aggregated PrP using  
8 steel powder only enabled the diagnosis of vCJD but not other types of PrDs<sup>183,184</sup>. Thus, the definitive  
9 diagnosis of PrD currently still depends on the analysis of brain samples, which, with a few exceptions  
10 involving brain biopsies, occurs postmortem. The presence of prion deposits can then be detected via  
11 immunoblotting or immunohistochemistry, and neuropathological changes such as gliosis, neuronal  
12 loss and spongiform changes can be visualized using immunohistochemistry.

13 Recently developed approaches have therefore concentrated on increasing the sensitivity of diagnostic  
14 tests by amplifying aggregates before detection. Protein misfolding cyclic amplification (PMCA)<sup>185</sup>,  
15 the amyloid seeding assay (ASA)<sup>186</sup>, and quaking-induced conversion (QuIC)<sup>187</sup> have been applied to  
16 verify the presence of minute amounts of prions in infected specimen from animals and human. The  
17 further development of the real-time QUIC (RT-QUIC) assay allowed the assessment of human CSF  
18 samples<sup>188</sup>, and has now reached 96% sensitivity<sup>189</sup> and 100% specificity<sup>188</sup> in diagnosing CJD. Lately,  
19 the PMCA assay has been adapted for blood-based diagnosis of vCJD<sup>190,191</sup>; however, further studies  
20 are needed to validate this assay, especially regarding its application to other PrDs.

## 22 Box 2 | **Protein-misfolding disorders affecting the nervous system**

23 Neurodegenerative disorders have been linked to a variety of different protein aggregates and thus  
24 belong to the broader category of protein-misfolding disorders (PMDs). PMD-implicated proteins  
25 include APP,  $\alpha$ -synuclein, C9orf72, FUS, Huntingtin, Tau, TDP-43, and PrP<sup>C</sup>. Triggers such as stress,  
26 age, or mutagenesis are thought to induce misfolding of these proteins into toxic oligomeric species<sup>142</sup>.  
27 Different diseases, even though sometimes caused by aggregates of the same protein, show a spectrum



of neuropathological and clinical symptoms, indicating the presence of multiple aggregate strains that exert toxicity in distinct manners. With the exception of multiple system atrophy (MSA), which affects primarily oligodendrocytes, protein aggregates are usually most toxic to neurons. Synaptic defects seem to be an early event in neuronal PMDs and have been shown to cause neurodegeneration. Moreover, specific neuronal subtypes in different brain regions show a selective vulnerability to the different aggregates<sup>129,192,193</sup>. This suggests that the expression of co-factors such as receptors or chaperones can modulate the aggregate-induced toxicity and highlights the importance of cell-type specific future studies to decipher the differential vulnerability. The scheme below illustrates which protein aggregates cause which neuropathological (P) and clinical (C) changes and the associated PMD.

**Figure 1 | The nucleation and fragmentation cycle of prions and prionoids.** Chaperones linked to protein synthesis (CLIPs) guide and oversee the correct folding of newly synthesized polypeptide chains<sup>142</sup>. Misfolded proteins, induced by triggers such as overexpression, mutations, stress or age, either re-fold with the help of heat-shock proteins (HSPs), undergo degradation, or aggregate into  $\beta$ -sheet rich oligomers, which are considered the most toxic aggregate species. Endoplasmic reticulum associated protein degradation (ERAD)<sup>107,110</sup> or HSP70-mediated ubiquitination target misfolded monomeric and oligomeric aggregates for proteasomal degradation. By contrast, HSP90 stabilizes oligomers<sup>142</sup>, which can then form higher-order structures such as protofibrils or fibrils by incorporating correctly folded and misfolded monomeric proteins. The fragmentation of higher-order structures produces new propagons that can re-initiate the nucleation-fragmentation cycle.

**Figure 2 | Structure of the prion protein and amino acid substitutions that have been linked to genetic prion diseases.**

**a.** Tertiary structure of the prion protein( PrP) deduced from an NMR structure<sup>21,194</sup>. The unstructured flexible tail (FT) at the N-terminus consists of two hydrophilic charge clusters , and the octapeptide repeat region. A hydrophobic core links the FT with the globular domain at the C-terminus, encompassing three  $\alpha$ -helices, two short antiparallel  $\beta$ -sheets and two glycosylation sites. The addition of a glycosyl

phosphoinositol modification at the C-terminus anchors the protein to the plasma membrane.

**b.** Schematic representation of PrP and amino acid substitutions linked to the genetic prion diseases Fatal Familial Insomnia (FFI), genetic Creutzfeldt-Jakob disease (gCJD) and Gerstmann-Straeussler-Scheinker Syndrome (GSS). Where applicable, the amino acid present at polymorphic residue 129 is indicated;<sup>33,195</sup>: bold text indicates the presence of methionine; italic text indicates the presence of valine; bold italic text indicates that the disease occurs irrespective of the amino acid residue at position 129 . The asterisk indicates the substitution of an amino acid with a stop codon, which results in a truncated version of the protein.

### Figure 3 | **Therapeutic approaches targeting protein aggregation.**

Several different approaches have been undertaken to develop therapeutics for PMDs and show promising results in animal models. a. Overexpression of HSP70 and inhibition of HSP90 increases the degradation of misfolded proteins..

b. Anti-prions, innocuous versions of PrP<sup>Sc</sup>, compete with the aggregates for the same substrate and result in the formation of novel innocuous aggregates .

c. Luminescent conjugated polythiophenes (LCPs) hyperstabilize aggregates, thereby interfering with the nucleation-fragmentation cycle and consequently reduce aggregate propagation.

d. Antibodies specifically recognize and bind aggregates and can lead to aggregate clearance via phagocytic cells, interfere with the aggregation process, or prevent aggregates from exerting toxicity.

## Glossary

### PRION DISEASES

(PrDs) A group of diseases caused by an infectious protein, which includes genetic, acquired and sporadic forms. PrDs have an overall incidence of 1-2 cases per million individuals per year.

### PRION

The agent causing transmissible spongiform encephalopathies (TSE). As originally defined, the term does not have structural implications other than that a protein is an essential component. Although it is now generally accepted that the prion consists largely of PrP<sup>Sc</sup>, prions are defined as a biological activity rather than a physical entity. Hence they can be measured by activity assays rather than by quantitating PrP<sup>Sc</sup>.

### AGGREGATES

In the context of this review, the term “aggregate” is used to denote the coalescence of misfolded proteins into highly ordered structures, typically resulting in the formation of fibrils.

### PROTEIN MISFOLDING DISORDERS

(PMDs) Disorders that are characterized by protein aggregates, which induce neurodegeneration if present in the brain.

### PROPAGON

The minimal propagating unit of a misfolded protein, defined by its capacity to self-replicate in vitro and/or in vivo. A propagon that can transmit from a host individual to another individual is called a prion.

### PRIONOIDS

Protein aggregates that can propagate and spread between cells, but for which transmissibility between individuals has not yet been demonstrated.

### POLYMORPHISMS

Any sites in the DNA sequence that are present in the population in more than one state.

### PENETRANCE

the percentage of individuals with a mutation that exhibit clinical symptoms. Most *PRNP* mutations are highly penetrant, meaning that most individuals with *PRNP* mutations develop PrD.

### PRION STRAINS

Entities associated with distinct biochemical and neuropathological profiles, translating into a spectrum of incubation periods and clinical signs. Crucially, strain-specific traits are stable across serial transmission among isogenic hosts, indicating that they are encoded by the prion itself. Distinct structural assemblies of chemically identical PrP<sup>Sc</sup> are thought to underlie strainness.

### PHASE DEMIXING

Process of membraneless compartmentalization. Spontaneous demixing of two coexisting phases is driven by inter-molecular interactions, a propensity that seems to be particularly high for proteins with low-complexity domains.

### EXCITOTOXICITY

Neuronal overstimulation caused by increased levels of the excitatory neurotransmitter glutamate leading to calcium overload and mitochondrial dysfunction, and ultimately to neuronal cell death and memory loss.

### PARABIOSIS

Surgical technique to anatomically connect two individuals. The shared circulatory system between the individuals allows specific factors to be assessed for their involvement in regulating physiological functions, behavior, and disease pathogenesis.

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12  
13 ToC blurb

14 Parallels are increasingly being drawn between prion diseases and other aggregate-mediated  
15 neurodegenerative disorders. While prion diseases are a distinct subclass of protein misfolding disorders  
16 (PMDs), a better understanding of shared mechanisms is likely to benefit treatment of all PMDs.  
17  
18